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THE ROLE OF MICROORGANISMS IN MARINE FOULING AND BORING PROCESSES

By
Ralph Mitchell and Anthony M. Candell
assisted by
P.J. Boyle and T.D. Sleeter

Technical Report No. 3

February, 1977

Division of Engineering and Applied Physics
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report describes investigations conducted in our laboratory over the past year on the role of microorganisms in marine fouling and boring processes. The microbial succession on wood exposed to the sea was determined using scanning electron microscopy. The sequence in the succession was bacteria, pennate diatoms, cellulolytic bacteria, ciliated protozoa, and stalked protozoa. Macroalgae and invertebrates, including barnacles and wood-boring mollusks and crustaceans, colonized the wood after the microorganisms were established. Impregnation of wooden discs with tannic acid

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The possible interaction between marine microorganisms colonized wood exposed in the sea and molluscan wood borers was studied. The presence of a bacterial film on the wood surface but not fungal growth or cellulase treatment increased the density of Lyrodus pedicellatus boring into the wood.

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1. Abstract

This report describes investigations conducted in our laboratory over the past year on the role of microorganisms in marine fouling and boring processes. The microbial succession on wood exposed to the sea was determined using scanning electron microscopy. The sequence in the succession was bacteria, pennate diatoms, cellulolytic bacteria, ciliated protozoa, and stalked protozoa. Macroalgae and invertebrates including barnacles and wood-boring mollusks and crustaceans colonized the wood after the microorganisms were established. Impregnation of wooden discs with tannic acid was found to prevent boring activity in field trails. Treatment of the tannin-impregnated wood with a ferric chloride solution bound the tannin within wood and increased its efficacy. In marine ecosystems, tannin was responsible for the macrozonal distribution of microbial epiphytes on the surface of the brown macroalga Ascophyllum nodosum and delayed the decay of senescent Rhizophora mangle leaves within the water column of a south Florida estuary. Scanning electron microscopy and chemical analyses of the red mangrove leaves demonstrated that leaching and biodegradation of the leaf tissue increased its palatability to marine invertebrates and fish. Decayed mangrove leaves support the detrital food web within the estuary.

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2. Introduction

Since bacteria are the initial colonizers of surfaces exposed to the sea, a knowledge of their nature, distribution, and interactions with fouling and boring organisms may provide the key to the control of marine fouling and boring processes. Past work in this laboratory has shown that primary bacterial film formation can be prevented by negative chemotaxis of the marine bacteria with a variety of organic compounds including tannic acid. These repellents when applied in paint to the surface of metal plates or impregnated into wood inhibit the growth of bacteria, algae and wood borers.

During the past year we have extended our research to gain an insight into the biological succession on wood discs placed in the sea, modified the tannic acid treatment procedure to prevent tannin from being leached from the wood and examined two natural microbial populations that are influenced by tannin. Preliminary experiments with wood discs incubated with marine microorganisms or treated with cellulase and then exposed in an aquarium containing wood borers has shown that a well established bacterial population on the wood surface will accelerate the settling and boring activities of molluscan wood borers.

3. Microbial Succession on Wood Exposed to the Sea

Since the importance of the growth of marine bacteria on solid surfaces exposed to the sea was first recognized by ZoBell (1), considerable work has been undertaken on the microbial succession on immersed glass surfaces (2, 3, 4) and other structural materials (5, 6). Bacteria are usually implicated in primary film formation. Periphytic communities colonize these surfaces aided by extracellular mucopolysaccharides laid down by the bacteria (7, 8, 9). With a wooden surface, colonization by cellulolytic bacteria and fungi will be expected within a heterotrophic succession. The role of cellulolytic microorganisms in marine boring processes remains problematic (10). During the past year we extended our research to determine the sequence of the microbial succession on wood placed in a subtropical coastal environment in order to gain a greater understanding of marine boring and fouling processes.

Wood discs were suspended in the water in an embayment adjacent to the Nova University Oceanographic Laboratory, Fort Lauderdale, Florida in December 1975. Wood sections were cut from the discs on two separate visits to obtain samples of the wood surface after 16 and 36 hours, 1, 3, 6 and 12 weeks after immersions. The nature and mode of attachment of bacteria, and other periphytic marine organisms was determined using a scanning electron microscope.

The presence of teredine borers and marine isopods attacking the wood discs was demonstrated by x-ray analyses after 6 and 12 weeks exposures in the sea.

The dominant organisms colonizing the wood with increasing exposure time in the sea are listed in Table 1. During the first day after submersion individual bacteria settled on the wood and laid down filaments of bacterially produced polymer to anchor themselves to the surface. Also bacteria tended to exploit the irregularities in the wood surface to colonize the wood. Signs of organics accumulating at the surface and aiding the attachment of bacteria was apparent. As had been illustrated by Paeral (11), populations of bacteria associated detritus are clearly attached to particles by filaments or are embedded in a matrix of polymer. Examples of bacteria displaying the mechanisms, of attachment described by Marshall (3, 7) when he worked in our laboratory are shown in Figure 1.

After a week in the water copious amounts of bacterial polymer covered the wood surface and attached pennate diatoms were common. Cellulolytic bacteria of the genera Cytophaga and Pseudomonas could be readily isolated in enrichment culture on a modified Kadota medium with filter paper as the sole carbon source. Close inspection of the wood surface showed that bacteria appeared to be perforating the wood as they were recessed into the surface as if they were exhibiting cellulolytic activity.

Thick piles of pennate diatoms, bacteria and detritus developed on the surface after 3 weeks exposure. Stalked diatoms colonized the wooden discs and beneath the microbial film the wood was being eroded by cellulolytic bacteria. In some fields of view, many of the pennate diatom were showing signs of degradation; the absence of bacteria on the collapsing diatoms suggested that the process of decay was one of autolysis (Fig. 2).

TABLE 1. Dominant Perphytic Organisms on the Wood Surface with Increasing Exposure Time

Days Immersed in the Water	Dominant Organisms
1	Bacteria
3	Bacteria and detritus
7	Bacteria and pennate diatoms
21	Cellulolytic bacteria, bacteria, pennate diatoms, stalked diatoms and amoeboid and ciliated protozoa
42	Cellulolytic bacteria, other heterotrophic bacteria, pennate diatoms, macroalgae, and molluscan wood borers
84	Cellulolytic bacteria, pennate diatoms, macro algae, colonial protozoa, barnacles, molluscan wood borers and wood-boring isopods.

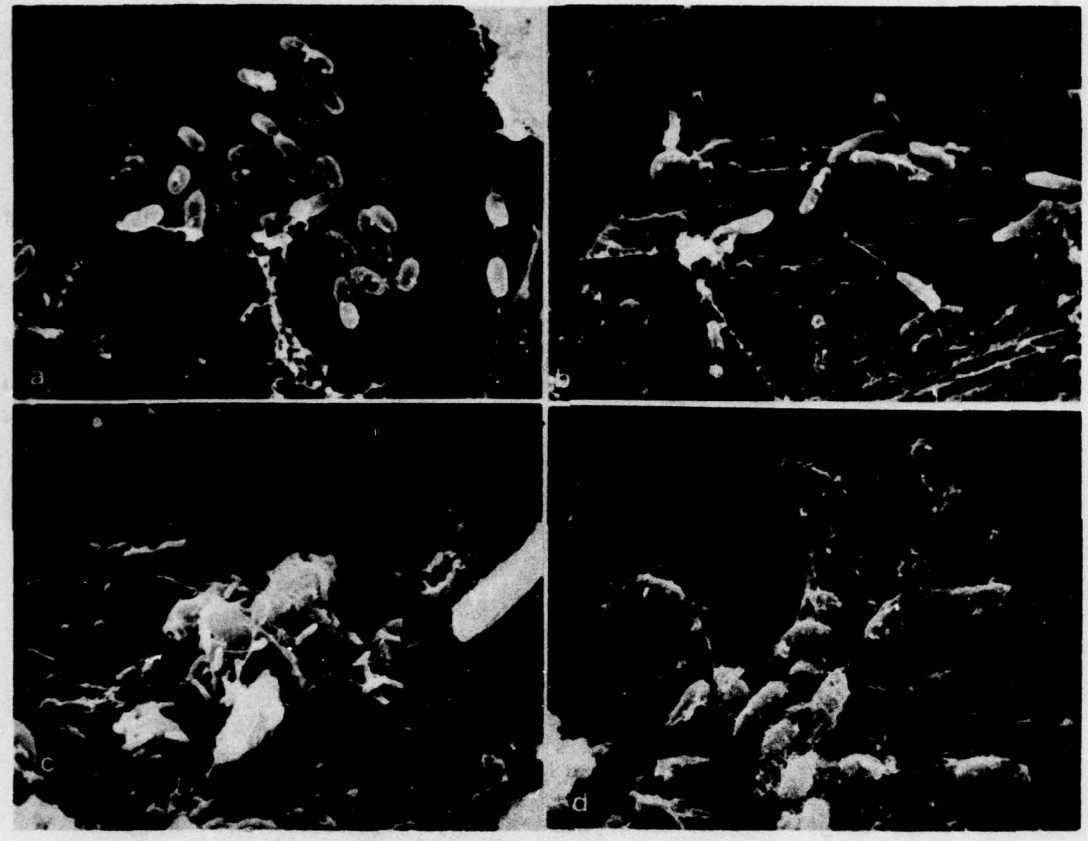

Fig. 1. Scanning electron photomicrographs of bacteria attached to the wood surface after 16 and 36 hrs. immersion in the sea.
(a) Microcolony of dividing rod-shaped bacteria and a stalked bacterium. Magnification 10,000X.
(b) Rod-shaped bacteria exploiting irregularities in the wood surface for attachment, note the anchoring filaments of polysaccharide. Magnification 10,000X.
(c) Bacteria associated with detrital material attached to the wood surface, note the large spirillum. Magnification 5,000X.
(d) Bacteria emeshed in copious amounts of polymer. Magnification 10,000X.

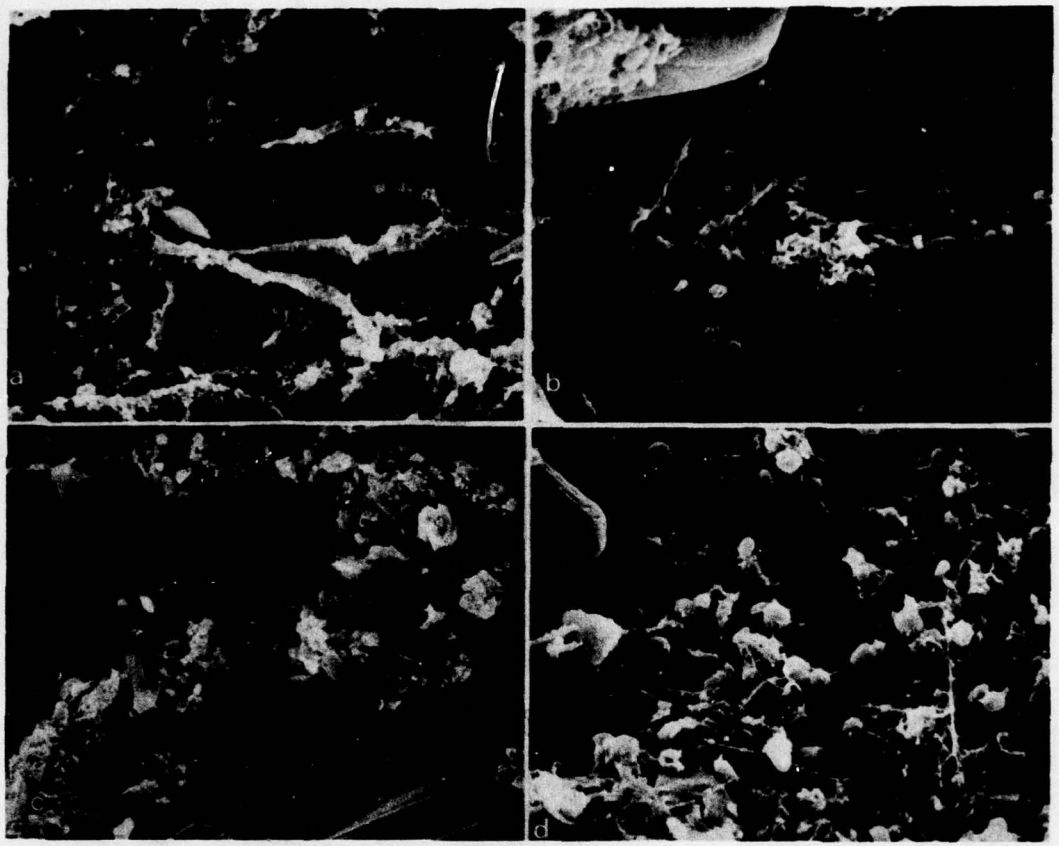

Fig. 2. Scanning electron photomicrographs of the wood surface after 1 and 3 weeks immersion in the sea.
(a) Attachment of pennate diatoms to the wood surface. 1 week exposure. Magnification 2,000X.
(b) Initial attack of the wood by cellulolytic bacteria. Note some bacteria are recessed into the wood surface. 1 week exposure. Magnification 10,000X.
(c) Thick piles of bacteria and pennate diatoms. 3 weeks exposure. Magnification 5,000X.
(d) Marked attack of the wood surface by cellulolytic bacteria. 3 weeks exposure. Magnification 10,000X.

Considerable honey-combing of the wood surface occurred after 6 weeks in the embayment. Diatoms were continuously being lost and replaced from the wood surface. The macroalgae such as the green seaweed Ulva appeared at this time. The bacterial film was observed to be grazed by protozoa. Examples of ameboid and ciliated protozoa were obtained from wood samples maintained in an aquarium to illustrate the diversity of these bacteria-grazing organisms (Fig. 3).

After 12 weeks a crust of partially digested wood was on the wood surface. When a wood sample to be prepared for scanning electron microscopy was cut from the disc, part of the surface was sheared exposing the underlying wood. Observation showed that cellulolytic bacteria penetrated the interior of the wood (Fig. 4); also prominent were marine invertebrates. In the vicinity of an attached barnacle larva which may have provided limited shelter were a number of protozoa of the genera Vortillium and Zoothamnium (Fig. 5). Limited signs of boring activity by the marine isopod Limnoria tripunctata were apparent on visual inspection of the wood while x-ray analysis of the wood discs revealed an extensive network of calcium carbonate lined burrows and individual marine borers of the genus Teredo (Fig. 6).

In summary, the sequence in the microbial succession observed by scanning electron microscopy was adventitous individual bacteria, bacterial microcolonies, pennate diatoms, ciliated protozoa, cellulolytic bacteria, stalked diatoms and sessile protozoa. Macroorganisms such as macroalgae and marine invertebrates including barnacles, and wood-boring isopods and mollusks colonize the wood after the microbial succession. Similar patterns of colonization have been reported by

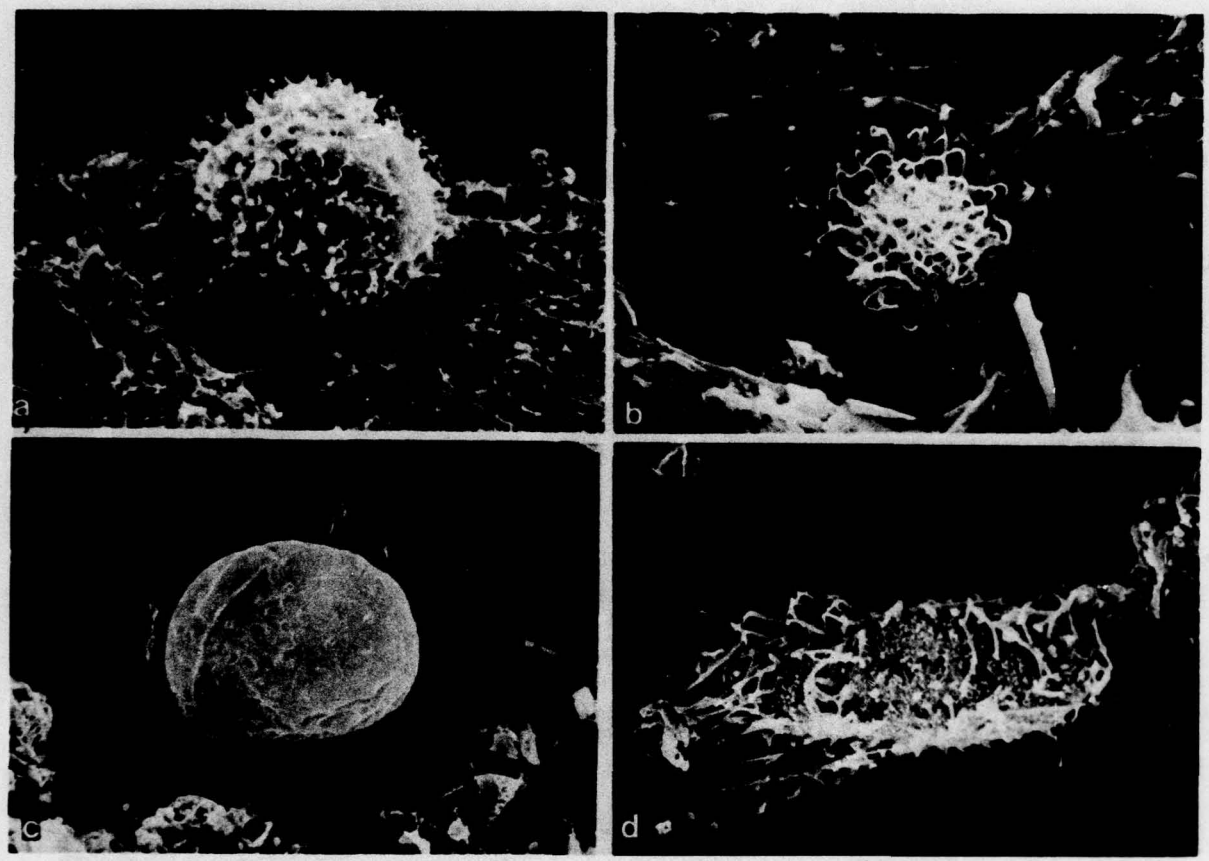

Fig. 3. Examples of ameoboid and ciliated protozoa grazing the wood surface.
(a) A denuded ciliated protozoan. Magnification 2, 000X.
(b) An ciliated protozoan. Magnification 3, 000X.
(c) An armored ameoba. Magnification 3, 000X.
(d) A ciliated protozoan. Magnification 4, 000X.

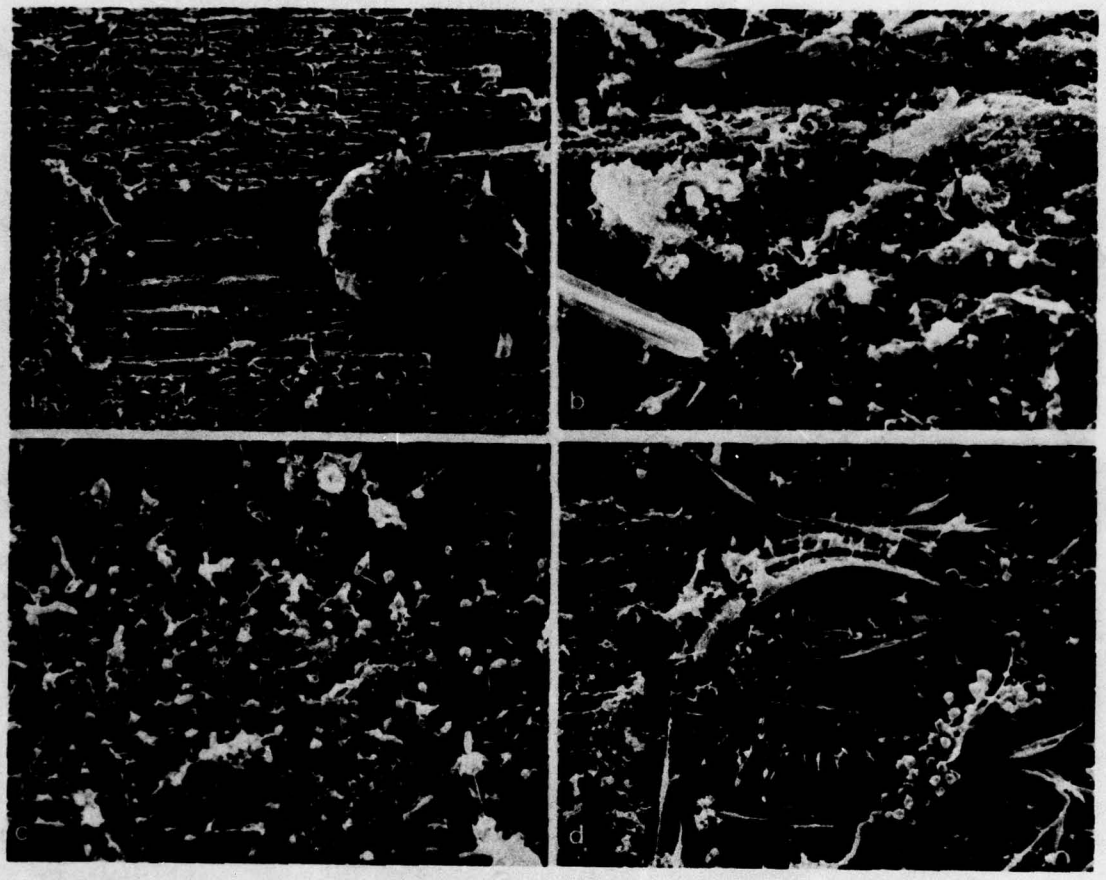

Fig. 4. Scanning electron photomicrographs of the wood surface after 12 weeks of exposure to the sea.
(a) Wood surface sheared back to expose the interior. Magnification 200X.
(b) Degraded outer surface. Magnification 1, 000X.
(c) Interior perforated by cellulolytic bacteria. Magnification 2, 000X.
(d) Barnacle larva providing shelter for stalked protozoa. Magnification 500X.

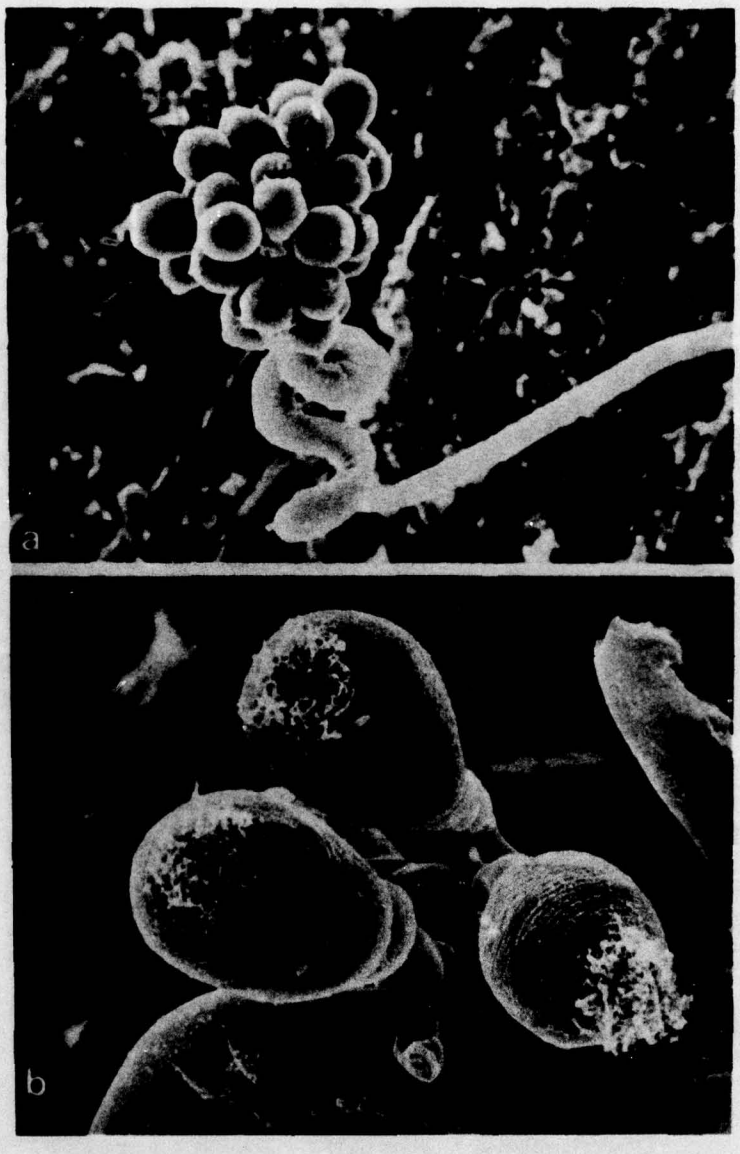

Fig. 5. Stalked protozoa of the genus Zoothamnium projected from the wood surface.
(a) Magnification 2, 000X.
(b) Magnification 10, 000X.

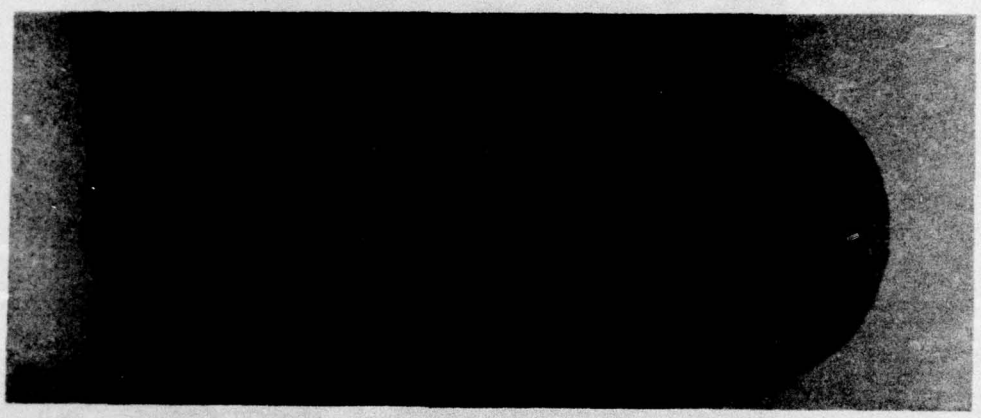

Fig. 6. X-ray photographs of the wood discs.
(a) 6 weeks of exposure.
(b) 12 weeks of exposure.

earlier workers. Notable was the absence of fungi on the wood discs. Cellulolytic bacteria were actively degrading the wood and may play a more significant role in facilitating the penetration of marine borers into the wood than cellulolytic fungi.

4. Use of Phenols to Control Marine Wood Boring Organisms

An initial field trial using wooden discs impregnated with 5% (W/V) tannic acid in 95% ethyl alcohol and a limited range of phenolic compounds was set up in early December 1975 in the embayment adjacent to the Nova University Oceanography Laboratory, Fort Lauderdale, Florida. The wood discs were inspected 12 weeks later and the amount of fouling on the surface estimated. The discs were cleaned and returned to the laboratory where x-ray pictures were taken of each disc to determine the extent of the borer damage. The results of the field trial are summarized in Table 2.

After 12 weeks in the water only tannin and pentachlorophenol were effective against wood borers. In addition, tannin was active against fouling. Impregnation of the phenolic compounds was followed by treatment with polyphenoloxidase to modify and perhaps polymerize the phenolic within the wood. The gallic acid-polyphenoloxidase treatment was then effective against molluscan wood borers whereas gallic acid alone was ineffective. The dopamine-polyphenoloxidase treatment was tried as it functions as a mechanism of fungal resistance in vascular plants like tomatoes (12) but it was ineffective and even stimulated Limnoria attack.

Inspection of tannin impregnated wood discs placed in the water six months earlier was revealing. The fouling of the surface was slight and no boring activity was observed. X-ray analysis confirm the absence of borer burrows within the wood. The wood had become considerably blackened. This color could be reproduced by staining the tannin treated wood with ferric chloride solution. $FeCl_3$ is a mild

TABLE 2. Extent of Fouling and Boring Activity on Wood Discs after 12 weeks in the Sea

Compound	Fouling	Molluscan Wood Borers	Wood-Boring Isopods
Control	Heavy fouling	Moderate attack	Light attack
Tannin	Slight fouling	No attack	Very light attack
Gallic acid	Heavy fouling	Heavy attack	Very light attack
Protocatechic acid	Heavy fouling	Moderate-light attack	Very light attack
Catechol	Heavy fouling	Moderate attack	Moderate attack
Quercetin	Moderate fouling	Moderate attack	Light-moderate attack
Gallic acid-polyphenol-oxidase (PPO)	Moderate-heavy fouling	Very light attack	Light attack
Catechol-PPO	Heavy fouling	Moderate attack	Moderate-heavy attack
Dopamine-PPO	Heavy fouling	Moderate-light attack	Heavy attack
Pentachlorophenol	Heavy fouling	Very light attack	Very light attack

oxidizing agent and forms dark colored complexes with tannin and other phenolic compounds. Since we made a general observation that tannin impregnated wood discs that turned black were less attacked than uncolored wood discs we decided that a ferric chloride treatment prior to submersion may be a most effective antiborer treatment.

A larger scale field trial was set up in July 1976. Wood blocks were used in the place of discs and a range of tannin impregnation concentrations were used with and without $FeCl_3$ treatment to determine the lower limit of the treatment. The following procedure was adopted:

1) The wooden block were dried at 110°C for 24 hours:
2) Placed in an impregnation press chamber and evacuated for 4-5 hours;
3) The tannin solution (5, 10, 20, or 40% W/V in ethanol) was drawn into the chamber;
4) The chamber pressure was maintained at 80 lb/in^2 overnight (16 hours);
5) The pressure was reduced slowly (30 minutes to an hour);
6) The blocks were airdried for a day;

and the cycle was repeated with 50% saturated $FeCl_3$ solution. In December, 1976 a trial with 20% tannin (W/V) impregnation with and without $FeCl_3$ complexing was placed in San Francisco Bay to test the efficiency of the treatment at another site.

Inspection of the surface of the tannin treated wood revealed the presence of abortive borer cones. Larvae of the shipworm Lyrodus pedicellatus at the pediveliger stage actively seek out the surface of wood place in aquaria. They settle on the surface randomly moving across the

wood until boring is initiated. Metamorphosis occurs and the Lyrodus bore into the wood surface forming a calceous cone. Abortive borer can be found on tannin impregnated wood suggesting tannin exhibits an obtusaquinone effect. Further work will be undertaken to clarify whether tannin influences the chemotactic response of molluscan wood borer larvae to wood.

Further trials with painted panels with and without tannin are underway in Fort Lauderdale, Florida and San Francisco Bay, California to reexamine the effectiveness of tannin as an antifouling agent. The difficulties of obtaining uniform dispersion of tannic acid in polyurethane and alkyld resin paints were overcome by dissolving tannic acid in minimal amounts of absolute ethyl alcohol. Sufficient tannic acid in 20 ml of alcohol was added give a final concentration of 10% tannin (W/W) in 40 g of paint. More attention has been given to the surface preparation of the stainless steel panels included polishing the surface, uniformly abrading the surface and rounding the edges of the panels to achieve excellent adhearance of the primer.

5. Tannins in Marine Ecosystems

a. Microbial Epiphytes on the Brown Alga Ascophyllum nodosum

The distribution of epiphytic microorganisms on the brown macroalga Ascophyllum nodosum collected from the intertidal zone of a rocky shore on the New England Coast was investigated. Seasonal changes in the diversity and biomass of the epiphytes have been related to the secretion of photosynthetically-produced mannitol, tannin content, ambient light intensity and temperature (13, 14, 15). We were most interested in the influence of tannin secretion on the distribution of microbial epiphytes on the Ascophyllum since tannin has potential as an antifouling and boring agent.

The nature and distribution of microbial epiphytes were determined using scanning electron microscopy. The number of bacterial cells per unit area of the seaweed can be determined by counting the number of bacteria within a number of scanning electron photomicrographs. Fields of view of the seaweed surface were randomly selected, photographed at a magnification of 10,000 times, the bacteria counted and number per cm^2 determined.

The amount of tannin-like compounds secreted from the surface of individual Ascophyllum seaweeds and from the apical tips, middle section of the main steam and the holdfast region was determined in the field using a Hach portable colorimeter. An average of 2 mg of tannin per hour per Kg of wet seaweed was secreted into the water column. This represents a turnover of 5% of the wet weight of the seaweed per day. Measurements with sections cut from the middle of the main stem, the

apical tips and holdfast from a number of individuals and sealed with molten paraffin wax showed that the respective rates of tannin secretion from the apical tips and holdfast region was 4 and 10 times greater than the middle region.

Differences in the microbial populations on the holdfast internodal regions of the main stem representing four years of growth and the apical tips were apparent. The populations ranged from a lawn of end-attached rod-shaped bacteria on the main stem above the holdfast to microcolonies of yeast near the apical tips. The greatest diversity of microorganisms was noted in the internodal region representing the fourth year of growth were a dense lawn of end-attached bacteria was overlaid by filamentous bacteria, pennate diatoms and filamentous blue-green algae. It is notable that this region had the least amount of tannin secretion.

The surface adjacent to the holdfast was irregular, mineral encrusted and colonized by low numbers of end-attached rod-shaped bacteria (Fig. 7). These bacteria cannot be described as stalked in the conventional sense as are Caulobacter since their mode of attachment resembles that described for Pseudomonas and other gram-negative motile rods (3, 16).

The first float appears on Ascophyllum after 2 years of growth. In the region representing the third year of growth, the bacterial lawn was complete with the filamentous bacterium Leucothrix mucor projecting from the seaweed surface (17). A few slender strands of Flexibacterium were found in each field of view (Fig. 8).

In addition to the bacterial epiphytes pennate diatoms and filamentous blue-green algae colonized the surface of Ascophyllum in the internodal

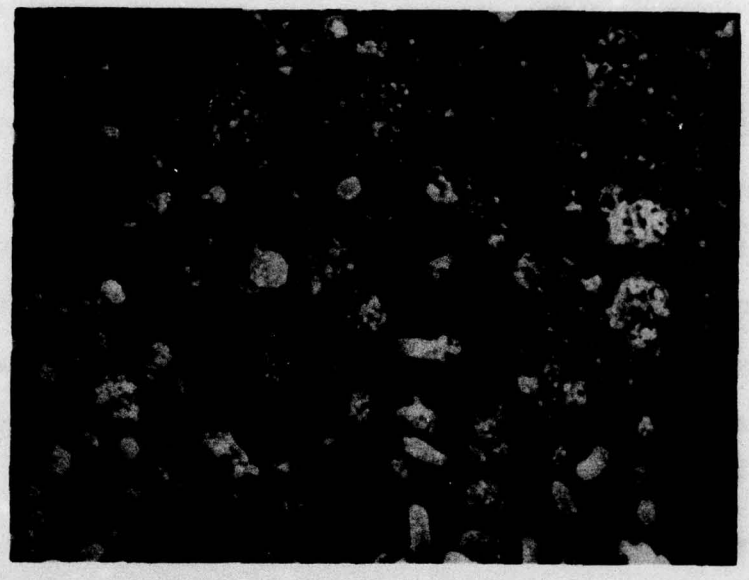

Fig. 7. Scanning electron photomicrograph of end-attached rod-shaped bacteria on the holdfast of the brown macroalga Ascophyllum nodosum. Magnification 10,000X.

Fig. 8. **Scanning electron photomicrograph of the bacterial lawn on the main stem of Ascophyllum nodosum. Third year of growth. Magnification 5,000X.**

region representing the fourth year of growth. A bacterial lawn of end-attached rod-shaped bacteria was covered with an abundance of flexibacteria and pennate diatoms of the genera Cocconeis and Nitzschia and interspersed with yeast microcolonies (Fig. 9) suggested an optimal light intensity, higher nutrient status and maturity within this region. The lower tannin secretion from the seaweed probably enabled the development of the diversity and biomass.

Nearer the apical region, the surface of the year-old tissue was colonized mainly by yeast microcolonies interspersed by end-attached bacteria and a limited number of pennate diatoms (Fig. 10). In sharp contrast to rich population of microbial epiphytes on the main steam, there was a complete absence of microorganisms on the apical tips of Ascophyllum. The secretion of tannin-like compounds, dessication during the tidal cycle, and the recent growth within the meristematic tissue probably prevented colonization of the seaweed surface.

Motile bacteria are attracted to algal surfaces by the exuded organic compound and localized concentration levels using chemotaxis. However, tannins produced by brown algae restrict the colonization of the seaweed surface by microorganisms. Recent investigations in our laboratory showed that tannic acid produced a negative chemotactic response from motile marine bacteria (19), at concentrations of the order found in seawater surrounding Ascophyllum (1-2 ppm of tannin). These finding suggest that tannin secretion by the seaweed repels motile bacteria seeking out surfaces to colonize. Since more than 90% of the bacteria in seawater are motile negative chemotaxis can be considered a powerful detriment to bacterial colonization of surfaces exposed to the sea.

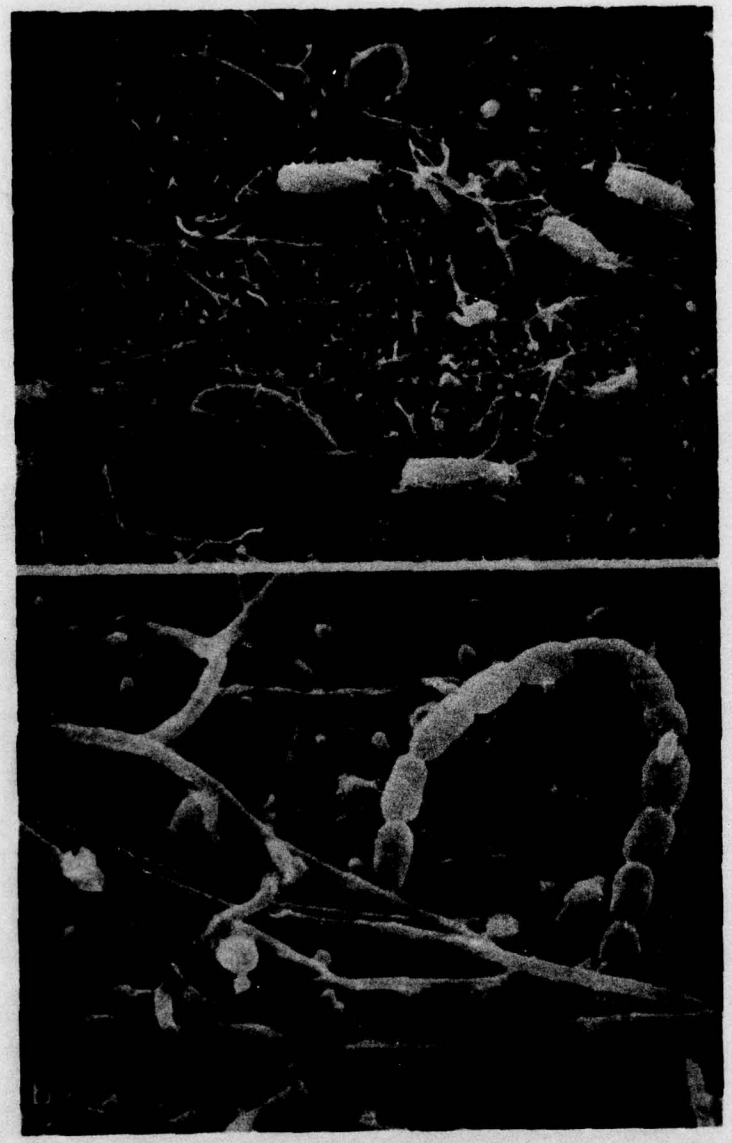

Fig. 9. Scanning electron photomicrographs of the microbial epiphytes on the main stem of Ascophyllum nodosum. Fourth year of growth.
(a) Microbial epiphytes include pennate diatoms, bacterial lawn of end-attached bacteria interspered with yeast cells and overlying flexibacteria. Magnification 2,000X.
(b) Same field. Note a probable filamentous blue-green alga. Magnification 10,000X.

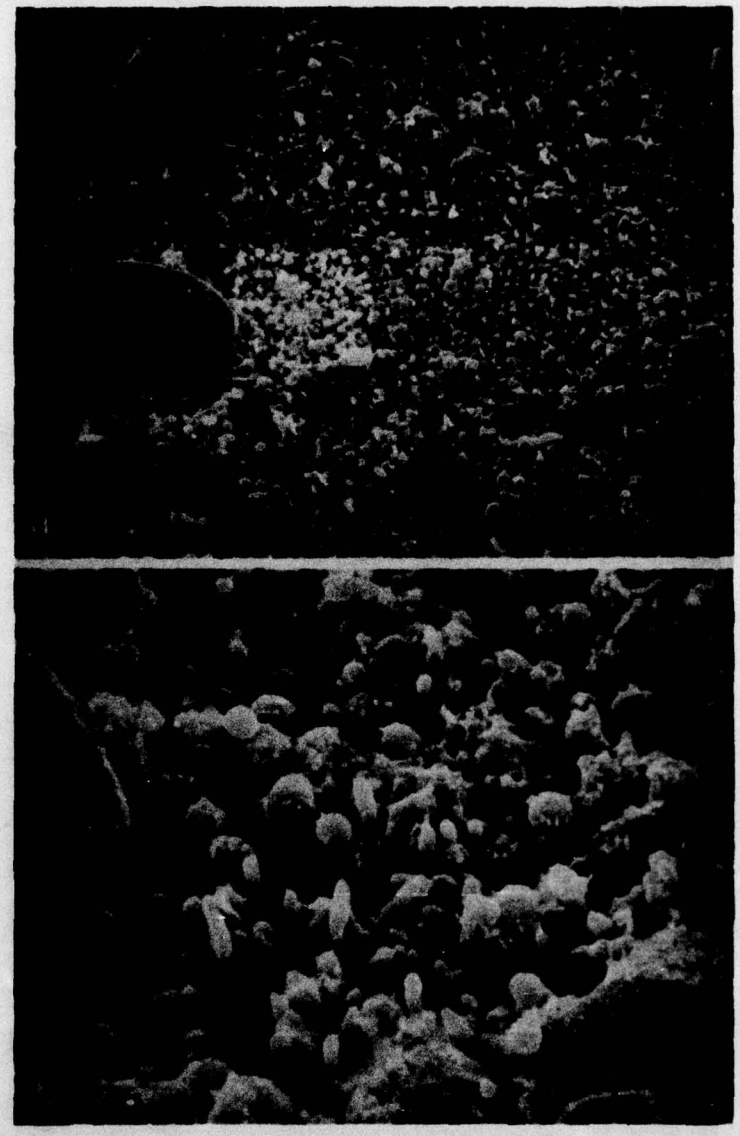

Fig. 10. Scanning electron photomicrographs of the microbial epiphytes on the brown macroalga Ascophyllum nodosum. Fifth year of growth.
(a) Pennate diatoms, rod-shaped bacteria and yeast microcolonies. Magnification 2,000X.
(b) Bacteria Magnification 10,000X.

b. Microbial Degradation of Red Mangrove (Rhizophora mangle) Leaves

Detrital food webs in the estuaries of South Florida are based largely on leaf-falls from the red mangrove Rhizophora mangle. The organization of the food web suggests that microorganisms degrading the structural material of the mangrove leaves support the populations of detritus consumers including invertegrate species and fish (20). Earlier work by Heald (21) demonstrated that red mangrove leaves exposed in fine mesh nylon bags lost 95% of their material in a year in brackish water. In the first 2 months, tannins were believed to diffuse out of the leaves and bacteria and fungi attacked the leaf material. The decrease in carbon: nitrogen ratio associated with the microbial growth at expense of the leaves made the leaves more palatable to detritus consumers. More recent investigations emphasized the sequence in fungal colonization, alternations in dry weight, carbon and nitrogen contents of the mangrove leaves contained in nylon bags anchored to the mangrove roots and litter collecting on the bottom (22) and the nitrogen fixation associated with the decaying leaves (23).

Since the mangrove leaves contain high concentrations of tannins we were interested in the influence these chemicals have on the decay of the leaves. This report will discuss changes in carbon, nitrogen, reducing sugar and tannin content of mangrove leaves suspended in the water column of a south Florida estuary. The microbial populations associated with the decomposition of the leaves during the first 70 days of exposure was determined using scanning electron microscopy.

Senescent red mangrove leaves suspended in the water column in a cove on Virginia Key near Miami, Florida were retreaved from the

fine mesh nylon bags after regular time intervals up to 70 days and were prepared for scanning electron microscopy using techniques standard to this laboratory. Chemical analyses and caloric content determination were conducted on milled leaf material. During the first 70 days submerged in the water column the carbon content decreased from 46.2 to 36.2% of the leaf material while the nitrogen content increased from 0.5 to 0.9%. This change represented a decrease in the carbon: nitrogen ratio from 92.4 to 40.2. This increase in nitrogen in decaying leaves has been observed by other investigators and represents the formation of microbial protein. The rate of nitrogen increase was 57 μg of N per g of mangrove tissue per day. Nitrogen-fixing organisms such as purple photosynthetic bacteria may contribute to this increase (23).

Readily leachable carbohydrates and tannins were lost from the leaf material by the 14th and 30th day, respectively. Reducing sugar increases within the leaf tissue during the first week in the sea and probably is due to autolysis. The tannin content of the senescent red mangrove leaves was 5.2%. The relative amount of tannin within the leaves increased day 3 through 7 to a peak of 8.4% and then declined to below 1% by day 35 (Fig. 11). As tannin is a well known enzyme inhibitor and antimicrobial agent the colonization of the leaves by bacteria and fungi is probably delayed by the leaching of tannin from the mangrove leaves.

The surface of the harvested senescent mangrove leaves was examined with the scanning electron microscope and the topside and the underside of an individual leaf could be identified by virtue of the presence of stoma on the underside of the leaves. A thick layer of amorphous waxy material covered the surface and sharp crystalline

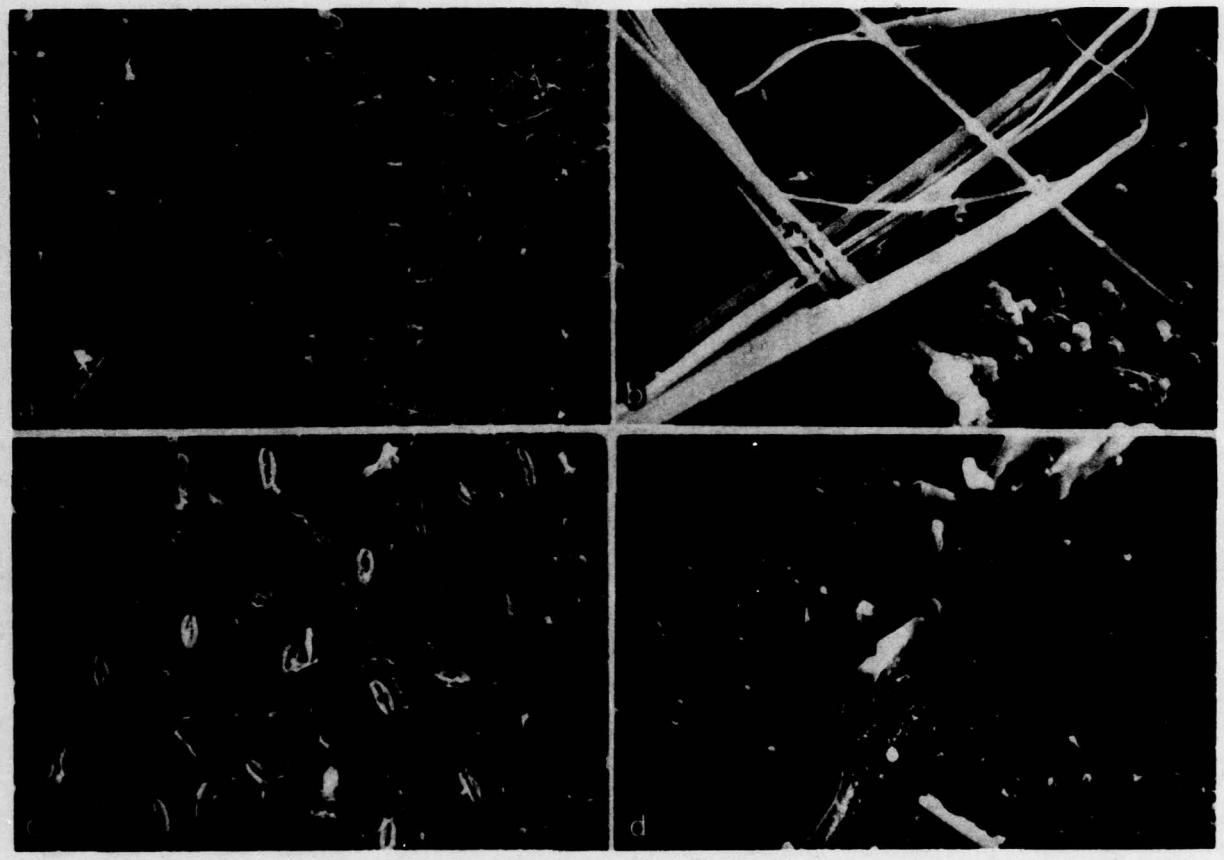

Fig. 11. Scanning electron photomicrographs of the leaf surface of Rhizophora mangle.
(a) Topside of a mangrove leaf. Magnification 200X.
(b) Needle-like deposits on leaf surface. Magnification 10,000.
(c) Underside of a mangrove leaf. Note the stoma and peeling cuticle. Magnification 200X.
(d) Peeling cuticle overlying the epidermis. Magnification 10,000X.

needles probably tannin were noticeable (Fig. 12). The colonization of the leaf surface by microorganisms was delayed until 28 days of immersion. After 28 days, the epidermis of the mangrove leaves was observed to be perforated by cellulolytic bacteria and fungi. An area where the epidermis was sheared away exposing the underlying tissue revealed very limited penetration and colonization of the leaf tissue (Fig. 13). Photomicrographs of the outer surface of the leaves at 35 days exposure in the water column show the diversity of microorganisms on the surface. Pennate diatoms, dinoflagellates, stalked protozoa were common. By the 56th day considerable degradation of the leaves was observed with a rich microflora ramifying through the surface of the exposed tissue (Fig. 14).

Although a sizeable biomass of microorganisms had built-up on the leaf tissue by the 70th day, the structural integrity of the leaves was intact. As the microorganisms grow into the leaf tissue the carbon: nitrogen ratio will decrease and the mangrove leaves will become more palatable to the organisms of the detrital food chain.

In conclusion, senescent mangrove leaves placed in the water in a subtropical estuary are not initially colonized by microorganisms. During the first month autolysis of the leaf tissue leads to the rapid leaching of carbohydrates and tannin from the intact leaves. The leaching of tannin, the waxy cuticle and thick epidermis probably delay the colonization of surface of the leaves. After a month a rich microflora including bacteria and fungi develop and slow degrade the structural material of the mangrove leaves.

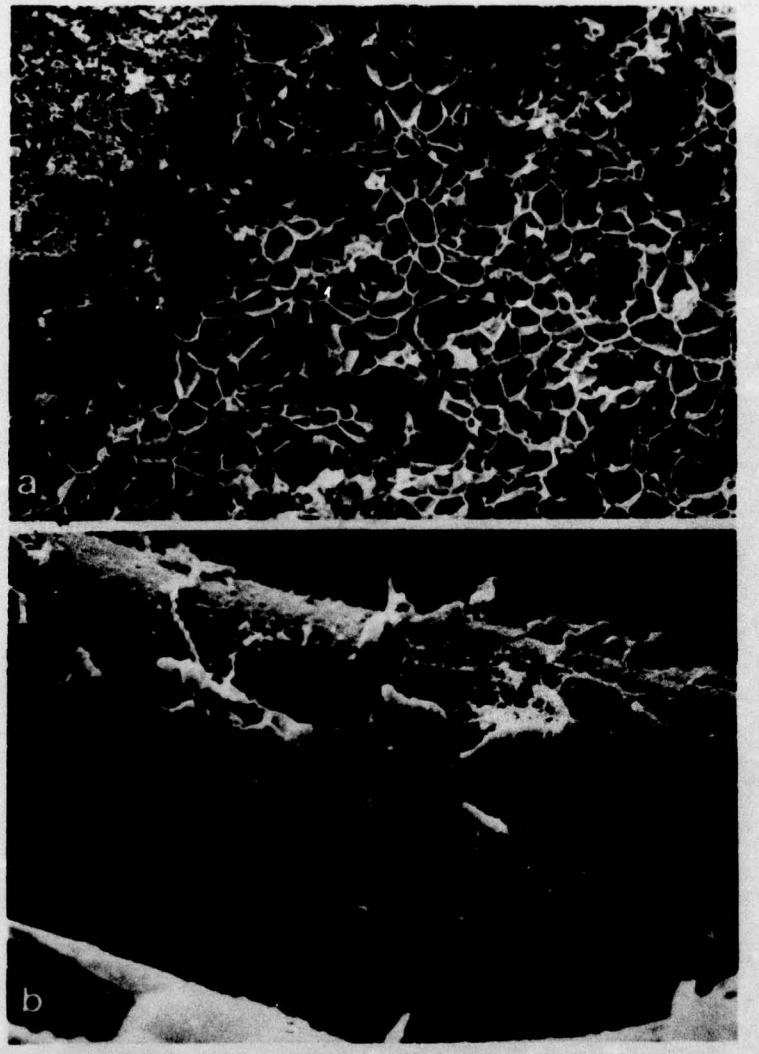

Fig. 12. **Scanning electron photomicrographs of the leaf surface of Rhizophora mangle after 28 days in the sea.**
(a) Leaf tissue beneath the epidermis . Magnification 200X.
(b) Bacteria colonizing the leaf tissue, note the mode of attachment. Magnification 10,000X.

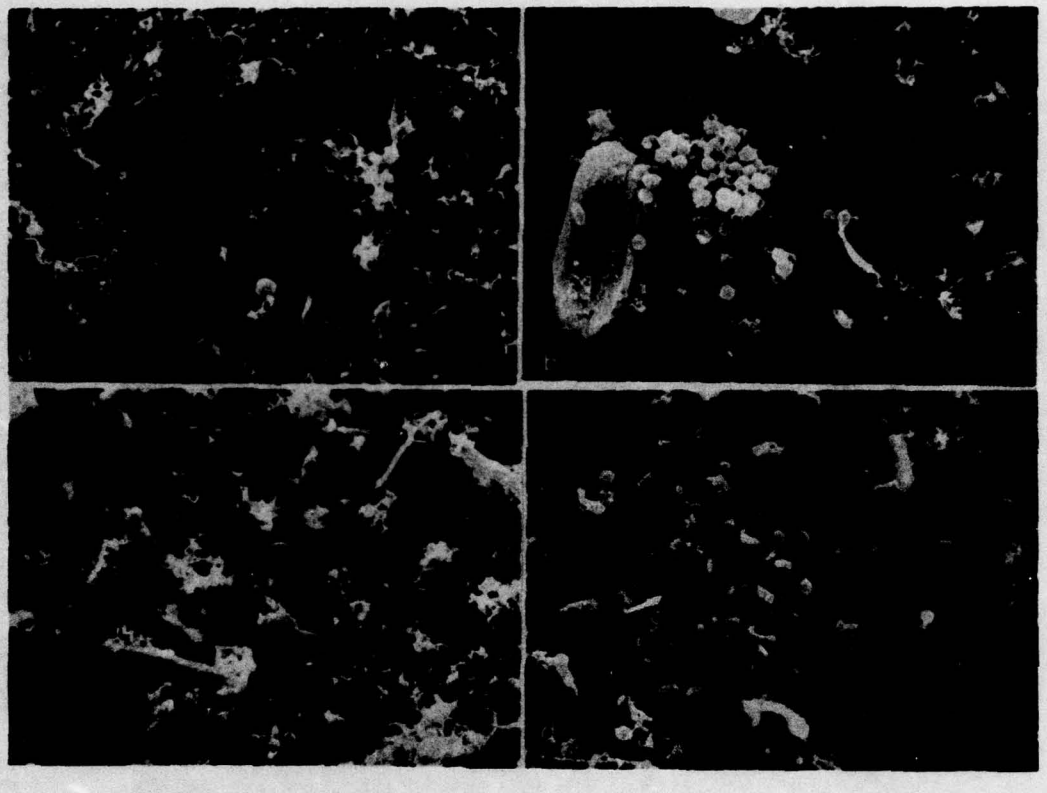

Fig. 13. Scanning electron photomicrographs of the leaf surface of Rhizophora mangle after immersion in the sea.
(a) Leaf surface after 35 days. Note the pennate diatoms, fungal mycelia, and stalked protozoa. Magnification 200X.
(b) Bacteria colonized the surface. After 35 days. Magnification 10,000X.
(c) Leaf surface after 56 days. Magnification 200X.
(d) Bacteria decaying the epidermis after 56 days. Magnification 10,000X.

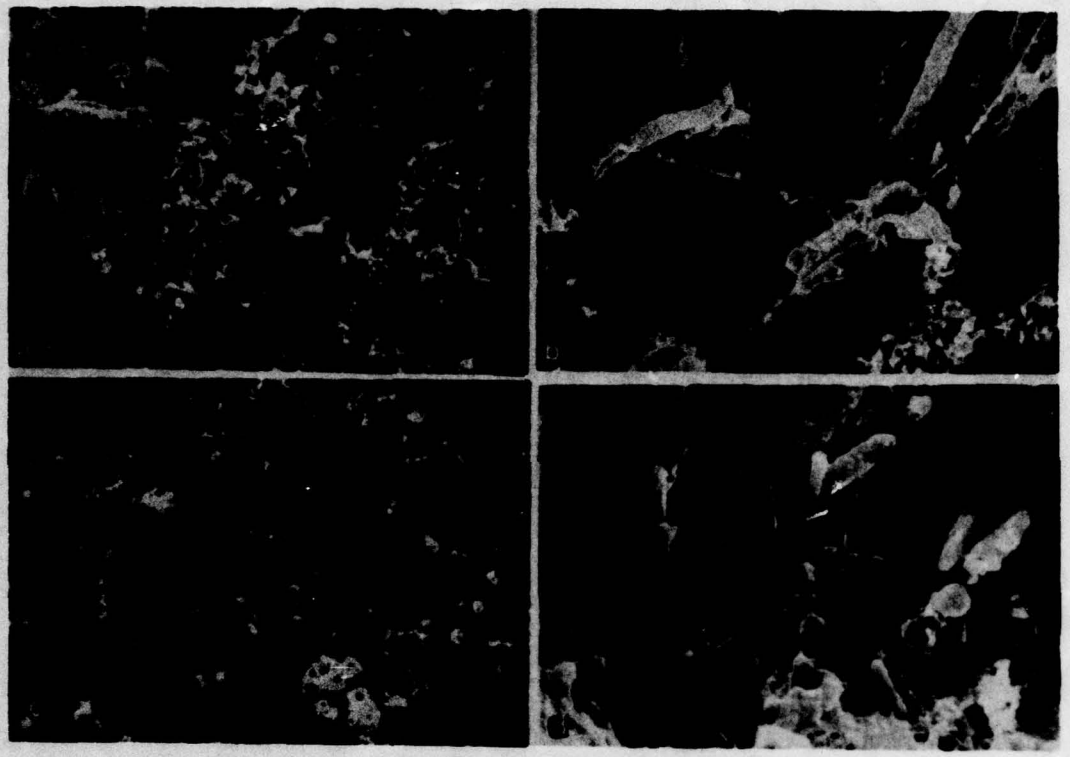

Fig. 14. Scanning electron photomicrographs of the leaf surface of Rhisophora mangle after 70 days in the sea.
(a) Population of tube worms, diatoms and fungi on the leaf surface. Magnification 200X.
(b) Fungal mycelia ramifying through the decaying leaf tissue. Magnification 10,000X.
(c) Decaying leaf tissue. Note that the epidermis is lost and colonies of diatoms are colonizing the tissue. Magnification 200X.
(d) Bacteria and possibly actinomycetes degrading the leaf tissue. Magnification 10,000X.

6. Interactions between Marine Microorganisms and Wood Borers

An experiment was designed to determine whether larvae of the teredine borer Lyrodus pedicellatus are attracted to, or preferentially settle on and bore into wood which supports a fungal or bacterial flora. Wood discs of dry southern pine (5 cm diameter, 0.7 cm thick) were incubated for 2 weeks on a shaking platform with a) A cellulolytic fungus Dendryphiella salina in a 0.1% yeast extract seawater medium, b) a sterile yeast extract seawater medium, c) an enrichment of marine cellulolytic bacteria in modified Kodato medium, d) a sterile modified Kodato medium, or e) impregnated with a homogenate of D. salina, f) impregnated with phosphate buffer, g) treated for 72 hours at 34°C with a solution of cellulase and h) incubated in sterile seawater. Triplicate samples of the treated discs where exposed in a flow-through marine aquarium maintained in Dr. R. D. Turner's laboratory in the Marine Biology Laboratory, Woods Hole, Massachusetts. The aquarium contained 2 large blocks of wood supporting a large population of mature Lyrodus pedicellatus. The numbers of shipworm larvae actively boring the surface of the wooden discs were counted.

The density of L. pedicellatus actively boring into the surface of the wood discs was determined after 18 days exposure (Table 3). The greatest density of wood borers was on the discs incubated with a mixed culture of marine bacteria including a cellulolytic Cytophaga sp. This treatment resulted in an attack significantly more numerous than the fungal treatments, digestion with cellulase and their respective controls. However, the bacterial control and the seawater soak was not significantly

different than the bacterial treatment. Further investigations are planned to explain these observations.

TABLE 3. Density of Lyrodus pedicellatus actively boring into the surface of the wood discs after 18 days exposure in an aquarium (sample size = 24)

Treatment	Density of L. pedicellatus per cm^2
Bacteria	9.8
Bacterial Control	6.9
Fungus (D. salina)	3.5
Fungal Control (n=6)	1.4
Fungal Extract	3.2
Buffer	4.0
Cellulase	4.6
Seawater	6.0

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